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Extensive Spinal Decussation and Bilateral Termination of Cervical Corticospinal Projections in Rhesus Monkeys

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Abstract

To examine neuroanatomical mechanisms underlying fine motor control of the primate hand, adult Rhesus monkeys underwent injections of biotinylated dextran amine (BDA) into the right motor cortex. Spinal axonal anatomy was examined using detailed serial-section reconstruction and modified stereological quantification. 87% of corticospinal tract (CST) axons decussated in the medullary pyramids and descended through the contralateral dorsolateral tract of the spinal cord. 11% of CST axons projected through the dorsolateral CST ipsilateral to the hemisphere of origin, and 2% of axons projected through the ipsilateral ventromedial CST. Notably, corticospinal axons decussated extensively across the spinal cord midline. Remarkably, nearly two-fold more CST axons decussated across the cervical spinal cord midline (~12,000 axons) than were labeled in all descending components of the CST (~6,700 axons). These findings suggest that CST axons extend multiple segmental collaterals. Furthermore, serial-section reconstructions revealed that individual axons descending in either the ipsilateral or contralateral dorsolateral CST can: 1) terminate in the gray matter ipsilateral to the hemisphere of origin; 2) terminate in the gray matter contralateral to the hemisphere of origin; or 3) branch in the spinal cord and terminate on *both* sides of the spinal cord. These results reveal a previously unappreciated degree of bilaterality and complexity of corticospinal projections in the primate spinal cord. This bilaterality is more extensive than that of the rat CST, and may resemble human CST organization. Thus, augmentation of sprouting of these extensive bilateral CST projections may provide a novel target for enhancing recovery after spinal cord injury.

Keywords

Spinal cord; Macaque; Primate; Motor neuron; Stereology; Anatomy

Introduction

The corticospinal system is critical for motor function in higher primates. Complete bilateral pyramidotomy in macaques, which transects the corticospinal tract (CST) but spares

bulbospinal and ascending sensory tracts, causes permanent loss of independent finger movements, but only transient loss of more gross aspects of motor control, such as reaching and locomotion (Hepp-Reymond et al., 1974; Lawrence and Hopkins, 1976; Lawrence and Kuypers, 1968). Evolutionarily, increased prominence of direct corticospinal connections to the motor pool corresponds with greater digital dexterity (Bortoff and Strick, 1993; Harting and Noback, 1970; Heffner and Masterton, 1975; Kuypers, 1964). Both anatomical and electrophysiological evidence supports the existence of direct projections of upper motor neurons to lower motor neurons innervating hand and finger muscles in various species of primates (Bernhard et al., 1953; Bortoff and Strick, 1993; Darian-Smith et al., 1996; Dum and Strick, 1996; Fetz and Cheney, 1980; Fetz et al., 1976; Kuypers, 1981; Lawrence et al., 1985; Lemon, 1993; Phillips and Porter, 1977; Porter and Lemon, 1993; Ralston and Ralston, 1985; Rouiller et al., 1996). Moreover, developmental maturation of the CST in primates occurs at approximately the same time as the emergence of fine motor control (Kuypers, 1962; Lawrence and Hopkins, 1976; Lemon, 1993).

The primate CST possesses a small, but significant, bilateral component. A small number of primate CST axons do not decussate at the medullary pyramids, but descend ipsilaterally (Bortoff and Strick, 1993; Galea and Darian-Smith, 1997; Kuypers and Brinkman, 1970; Kuypers, 1981; Lacroix et al., 2004; Leyton and Sherrington, 1917; Liu and Chambers, 1964; Phillips and Porter, 1977; Porter and Lemon, 1993; Ralston and Ralston, 1985). Furthermore, CST axons originating from a single cortical hemisphere can terminate on either side of the spinal cord (Bortoff and Strick, 1993; Dum and Strick, 1996; Galea and Darian-Smith, 1997; Hoff and Hoff, 1934; Kuypers, 1960; Kuypers and Brinkman, 1970; Lacroix et al., 2004; Liu and Chambers, 1964; Ralston and Ralston, 1985; Tigges et al., 1979). One possible functional significance of this architecture is apparent after unilateral pyramidotomy, which specifically transects all CST axons originating in one cortical hemisphere, yet is followed by partial recovery of independent finger movements in the contralesional hand (Hepp-Reymond and Wiesendanger, 1972; Schwartzman, 1978). It is possible that the intact CST retains sufficient connectivity with the lesioned side of the spinal cord to support functional recovery; alternatively, the recovery could reflect the activity of intraspinal relays, including propriospinal projections (Alstermark et al., 2007; Isa et al., 2007).

However, critical anatomical mechanisms underlying bilateral corticospinal control of motor action, particularly at cervical levels, remain poorly understood. First, the proportions of CST axons in various descending tracts have not been quantified in the cervical region. In the present work, we used a stereologically-based quantification method to establish these proportions. Second, the degree of CST axon decussation across the cervical spinal cord midline has not been documented. In this study, we quantified the number of midline-crossing axons in spinal cord segments C3 and C8. Third, precise projection and termination patterns of individual CST axons have not been identified. Here, we serially reconstructed CST axons and their terminal arbors to directly define the precise anatomy of CST projections. We now report a remarkable degree of bilateral spinal cord projection and midline decussation of corticospinal axons originating from a single hemisphere in primates, highlighting a quantitative distinction from corticospinal organization in rodent species, with resulting implications for promoting neural plasticity after injury.

Materials and Methods

Subjects

Three rhesus monkeys (*Macaca mulatta*), aged 8, 15, and 5 years, were used as subjects in this study. The subjects are referred to herein as A, B, and C, respectively. Subjects A and C were male, and subject B was female. The monkeys had not been involved in surgical

procedures or pharmacological experiments prior to the present study. Subjects were housed and surgical procedures were performed at the California Regional Primate Research Center, Davis, CA. All surgery and animal care adhered to the American Association for the Accreditation of Laboratory Animal Care and institutional guidelines.

Tracer surgery

Subjects were sedated with 1 mg/kg ketamine intramuscularly (i.m.), then anesthetized with 1.5–2.5% isoflurane. After mounting in a stereotaxic frame, a frontoparietal craniotomy was performed to expose both the right motor cortex. At each of 127 injection sites (59 locations, many at multiple depths; Fig. 1) in the right primary motor cortex, approximately 150 nanoliters of lysine-fixable biotinylated dextran amine (BDA; 10% in H₂O; 10,000 molecular weight; Molecular Probes, Eugene, OR) was injected with a pulled glass micropipette (outside diameter at tip ~40 µm) attached to a picospritzer (Parker Hannifin Corp., Fairfield, NJ). These sites included areas of motor cortex that innervate: 1) the hand and arm: 84 injections were made at 28 locations, 1.5 and 2.5 mm anterior to the central sulcus, spanning a territory from 13.3 to 24.5 mm lateral to the sagittal sinus and at depths in each site of 2.0, 3.5 and 5.5 mm (Fig. 1); 2) the trunk: 11 injections were made at 11 locations, 1.5 mm anterior to the central sulcus, spanning a territory from 3.9 to 12.5 mm lateral to the sagittal sinus and at a depth of 2.2 mm (Fig. 1); 3) the foot and lower extremities: 32 injections were made at 20 locations, 2 and 3 mm lateral of the sagittal sinus, spanning a territory from 1 to 11 mm anterior to the junction of the central sulcus and the sagittal sinus and at depths ranging from 1.8 to 6.5 mm, (Fig. 1). After tracer injection, the craniotomy flap was replaced and the incision was closed.

Sacrifice and tissue processing

6–7 weeks after tracer injections, subjects were sedated with 1 mg/kg i.m. ketamine, then deeply anesthetized with intraperitoneal pentobarbital (25–30 mg/kg, to effect) and transcardially perfused for 10 minutes with 1% paraformaldehyde at 4° C (in 0.1 M phosphate buffer, pH 7.4), then for 50 minutes with 4% paraformaldehyde at 4° C, for a total perfusion volume of approximately 8 liters. The entire spinal cord was dissected from the spinal column, and the brain was exposed by a partial craniotomy. Both spinal cord and cranium were post-fixed in 4% paraformaldehyde for 5–6 hours, transferred to 10% glycerin (in 0.1 M phosphate buffer) for 12–24 hours, then placed in 20% glycerin. The spinal cord dura was removed, and the spinal cord was cut in the transverse plane into blocks 1–1.5 cm-long, using the nerve roots as a guide to spinal level. The block containing cervical segments 6 and 7 was sectioned in the horizontal plane on a freezing microtome set at 30 µm intervals. Blocks containing segments C3–C5 and C8–T1 were sectioned in the transverse plane on a freezing microtome set at 40 µm. Tissue sections were stored at –20° C in cryoprotectant (25% glycerin, 30% ethylene glycol in 0.5 M phosphate buffer). The brain was cut coronally, *in situ*, into 3 cm blocks. These blocks were dissected from the remaining cranium and placed in 20% glycerin. The block containing the motor cortex was sectioned coronally on a freezing microtome set at 40 µm intervals. Tissue sections were stored at –20° C in cryoprotectant.

Transverse sections from segments C3 and C8 (three sections per segment per animal), horizontal sections from segments C6–7 (every 24th section), and coronal sections from primary motor cortex (every 24th section) were selected for BDA labeling. In addition, 96 consecutive sections from segments C8–T1 were selected for BDA labeling for serial reconstructions in subjects A and B. Sections were processed with the avidin-biotin amplification method with peroxide as a substrate. Sections were washed in 0.1 M Tris-buffered saline (TBS; pH 7.4) three times for 10 minutes. Endogenous peroxidase activity was reduced with 0.6% hydrogen peroxide in TBS for 30 minutes. Sections were rinsed

three more times in TBS, then incubated at 4°C overnight in an avidin-biotin-peroxidase complex solution (Vectastain ABC Elite Kit, Vector, Burlingame, CA; 1:111 dilution). After several rinses in TBS, sections were placed in a solution of diaminobenzidine (DAB) and NiCl_2 for 3–15 minutes until a dark reaction product was observed. Following three final rinses in TBS, sections were mounted on gelatin-coated slides and air dried. For Nissl staining, some sections were then defatted in a 1:1 mixture of chloroform and ethanol, rehydrated, and placed briefly in thionin. All sections were then dehydrated, cleared, and coverslipped.

Quantification and reconstruction of CST axons

BDA-labeled axons of passage were quantified in the three major components of the corticospinal tract: the contralateral dorsolateral tract (contralateral to the injected hemisphere), the ipsilateral dorsolateral tract, and the ipsilateral ventromedial tract (see Fig. 4). Quantification was performed on an Olympus BX60 microscope equipped with an Olympus OLY-200 video camera, a motorized stage and stage controller (MAC2002-XYZ, Ludl Electronic Products, Ltd., Hawthorne, NY), and StereoInvestigator software (MicroBrightfield, Colchester, VT) using modified stereological methods (see below). Three regions of interest, corresponding to the three components of the CST mentioned above, were outlined under low magnification (4x objective). The tracts were then examined under higher magnification (60x oil objective) for axon quantification.

In the ventromedial tract, all BDA-labeled axons of passage were counted. In the contralateral dorsolateral tract, axons were counted at 30–40 sampling sites selected randomly by StereoInvestigator software, covering an average of $1.7 \pm 0.5\%$ of the tract. In the more sparse ipsilateral dorsolateral tract, axons were counted at 120–135 sampling sites, covering an average of $6.6 \pm 1.8\%$ of the tract. Each sampling site (counting frame) was a $50 \mu\text{m} \times 50 \mu\text{m}$ square. To be counted, an axon had to: 1) be clearly labeled; 2) pass through the plane of the section (an axon of passage), rather than travel within the plane of section (thereby excluding axons exiting the white matter); and 3) have its profile on the upper face of the section within the inclusion zone delineated by the sampling software (see Fig. 2). To obtain an estimate of the total number of axons in the tract, the number of axons counted was multiplied by the inverse of the proportion of the tract area sampled (e.g., a sampling fraction of 2% produces a multiplier of 50). This quantification method has been modified from standard stereological methods, because the objects counted are cylinders passing through the plane of section, rather than spheroids contained within the section (Gundersen, 1986; Peterson, 1999; West, 1993). This quantification method is not subject to error caused by differential tissue shrinkage across subjects (Gundersen, 1986; Peterson, 1999; West, 1993). During quantification, each axon was categorized as ‘large’ ($\geq 3 \mu\text{m}$ dia.) or ‘small’ ($< 3 \mu\text{m}$ dia.).

BDA (Veenman et al., 1992) allows more sensitive visualization of fine fibers and terminal axonal arbors compared to tracers utilized in previous studies, including ^3H -leucine and wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). The use of BDA in these experiments permitted the detection of extremely fine descending and midline-crossing axons, as well as the serial reconstruction of axonal arbors (see below). The number of BDA-labeled axons crossing the spinal cord midline was counted in each section. Using StereoInvestigator, a dorsoventral line was drawn which bisected the gray matter at the central canal. The line extended from the dorsal border of the midline gray matter to the dorsal border of the ventral fissure. BDA-labeled axons that intersected this line were designated midline-crossing axons.

Partial serial reconstructions were performed on 7 selected BDA-labeled axons that crossed the spinal cord midline in the gray matter surrounding the central canal. 4 axons in Subject

A and 3 axons in Subject B were reconstructed using previously described methods (e.g., Havton and Ohara, 1993). Axons originating in the ipsilateral ventromedial tract were not reconstructed. In each subject, ninety-six consecutive transverse sections, collected in strict serial order, were examined with an E600 Nikon light microscope equipped with a reconstruction tube and a 100x oil objective. Labeled axonal processes and collaterals that exited one section were also identified in the adjacent section using three or more neighboring labeled structures to assure correct identification and orientation. No segments that could not be identified across sections were included in the reconstructions. Reconstructions began at the portions of the axons leaving the dorsolateral tract, tracked the axons as they crossed midline, and followed the axons to their terminal arbors. Some terminal arbors extended beyond the rostrocaudal span of the consecutive sections.

Image capture and manipulation

Images were captured using an Optronics Microfire A/R digital camera and PictureFrame software (www.optronics.com). Brightness and contrast were adjusted for optimal visibility using Adobe Photoshop CS2 (Adobe Systems, Inc., www.adobe.com). For low-magnification montages (Figs. 3, 4, and 5), component images were corrected for nonhomogenous lighting before being placed in the montage. This correction was performed in Adobe Photoshop CS2, using an image of an empty visual field taken under the same lighting conditions as the component images. This empty image was color-inverted, pasted into a new layer on top of each of the component images, and this layer was set to the 'color dodge' mode. The layers were then flattened, and the corrected image was pasted into a montage.

Composites of multiple focal planes (Figs. 6, 7, and 9) were constructed either in Adobe Photoshop CS2 or ImageJ 1.41c (Wayne Rasband, National Institutes of Health, rsb.info.nih.gov/ij/). For Fig. 7, individual images were taken from overlapping visual fields, hand-montaged in Photoshop, and overlying layers were set to the 'darken' mode (which allows the darkest pixels in the image stack to show through). For Figs. 6 and 9, all individual images were taken from the same visual field, then imported into ImageJ as an image sequence. The image sequence was then projected through the z-axis in 'minimum intensity' mode (identical to Photoshop's 'darken' mode).

All values reported herein are expressed as mean $\pm$ standard deviation.

Results

Examination of coronal brain sections revealed accurate targeting, in all subjects, of the primary motor cortex innervating the hand, trunk, and foot (Fig. 3). There were no qualitative differences between subjects in location or amount of tracer injected.

Quantification in transverse sections demonstrated that $87 \pm 3\%$ of CST axons projected through the contralateral dorsolateral portion of the spinal cord (Table 1 and Figs. 4a, 5a; 'contralateral' is relative to cortex receiving tracer injection). $11 \pm 3\%$ of CST axons did not decussate in the medulla, but descended through the cord in the ipsilateral (right) dorsolateral portion of the cord (Figs. 4b, 5b). The remaining $2 \pm 0.4\%$ of CST axons also descended ipsilaterally, in the ventromedial portion of the cord (Fig. 4c). Additional rare axons descended through the contralateral ventromedial CST and the dorsal columns. Axon diameters ranged from 0.5 to 5 μm (Fig. 6), with $19 \pm 7\%$ of the axons in the 3–5 μm range, and $81 \pm 7\%$ smaller than 3 μm . Subject C had fewer labeled axons than subjects A or B, likely attributable to a low molar ratio of lysine:dextran in the tracer lot used in this subject (average molar ratio of 1 lysine:dextran); because lysine residues link BDA to spinal cord tissue during fixation (Nance and Burns, 1990; Schmued et al., 1990), low lysine levels may

lead to loss of BDA during perfusion and subsequent tissue processing. Nonetheless, the proportion of axons in each tract of subject C was well within the range of the other two subjects.

Axonal projection along the rostrocaudal axis was not limited to the white matter. As revealed in both horizontal sections (Figs. 5d, 5f, 5g, and 7) and serial reconstructions (Fig. 8), some axons projected through the spinal cord gray matter for distances of up to 4 mm. CST axons branched either shortly before entering the gray matter, shortly after entering the gray matter, or well after penetrating the gray matter (Figs. 7 and 8).

A mean of 12 ± 9 axons decussated across the spinal cord midline within each transverse $40\mu\text{m}$ -thick section (Fig. 4d; subject A, 20 ± 9 axons; subject B, 13 ± 4 axons; subject C, 2 ± 1 axons). Mean measured section thicknesses did not differ across subjects (subject A, $51 \pm 9\mu\text{m}$; subject B, $54 \pm 9\mu\text{m}$; subject C, $52 \pm 4\mu\text{m}$). Most of the midline-crossing axons traversed the spinal cord midline in gray matter surrounding the central canal (Fig. 4d). Occasional axons also crossed the midline in white matter immediately ventral to Lamina X (Fig. 4d). Serial reconstructions revealed that axons crossing the midline originated from *both* sides of the spinal cord. Axons that crossed from the contralateral-to-ipsilateral side (relative to the hemisphere of origin) presumably crossed the midline twice, once in the medulla and again in the spinal cord (Fig. 8c). Axons that crossed from the ipsilateral-to-contralateral side, in contrast, did not decussate the midline in the medulla, but instead descended ipsilaterally, then crossed the spinal cord midline to form terminal arbors (Fig. 8a, b). When axons branched prior to crossing midline, in some cases both branches decussated across the midline (Fig. 8a). In other cases, remarkably, one branch formed a terminal arbor on the side of emergence from the dorsolateral CST, while the other axon formed a terminal arbor on the other side of the spinal cord (Fig. 8b, c). Thus, some cortical motor neurons extended terminal arbors that innervated the spinal cord bilaterally. These bilateral arbors were sometimes heterotopic (Fig. 8b) and sometimes homotopic (Fig. 8c).

Axonal termination patterns differed in the ipsilateral and contralateral spinal cord. Contralateral to the hemisphere of origin, CST axons terminated primarily in the intermediate zone of gray matter (Rexed's laminae V, VI, and VII; Figs. 4e, 5c, 5d), and to a lesser extent in laminae VIII and IX (Figs. 4f, 5e). Ipsilateral to the hemisphere of origin, CST axons terminated primarily in lamina VIII (Fig. 4c), and to a lesser extent in the intermediate zone (Figs. 4g, 5f), though some terminals were observed in lamina IX (Fig. 8c). Terminal arbors possessed both *en passant* and terminal bouton-like swellings (Fig. 9).

Discussion

This study reveals a remarkable extent of bilateral corticospinal tract projection and termination in the non-human primate spinal cord. This bilaterality is first apparent in the fascicular arrangement of the CST. That is, while 87% of CST axons descend in the dorsolateral tract of the spinal cord contralateral to the hemisphere of origin, another 11% of axons descend in the dorsolateral fasciculus that is ipsilateral to the hemisphere of origin. In addition, the bilaterality of the primate CST is apparent in the patterns of terminal axonal arborization. We have demonstrated a remarkable extent of corticospinal axon branching and decussation across the spinal cord midline. We observed a mean of 12 axons crossing the spinal cord midline in each transverse section of mean thickness $52\mu\text{m}$. Extrapolation suggests that approximately 12,000 BDA-labeled CST axons cross the spinal cord midline within the $\sim 5\text{ cm}$ cervical enlargement. This is a remarkably high number, given that a mean of 6,700 descending CST axons were labeled in the white matter in each subject, and that many of those labeled axons innervate the lumbar spinal cord, rather than the cervical spinal cord. Thus, nearly twice as many corticospinal axons decussate across the cervical spinal

cord midline as descend in the dorsolateral fasciculi of the spinal cord. This finding indicates both that corticospinal axons extend extensive collateral branches from their main projections, and that each hemisphere gives rise to bilateral terminals. Finally, through detailed serial-section reconstructions, we have found an unprecedented degree of bilaterality of terminal arbors in individual corticospinal axons. That is, some axons emerge from the dorsolateral fasciculus, bifurcate, and extend terminal arbors to both sides of the spinal cord.

The fact that a notable proportion of the CST descends and terminates ipsilateral to the hemisphere of origin is consistent with earlier neuroanatomical studies (Bortoff and Strick, 1993; Galea and Darian-Smith, 1997; Kuypers and Brinkman, 1970; Kuypers, 1981; Lacroix et al., 2004; Leyton and Sherrington, 1917; Liu and Chambers, 1964; Phillips and Porter, 1977; Porter and Lemon, 1993; Ralston and Ralston, 1985). However, all of these studies (except for Lacroix et al., 2004, in the lumbar spinal cord) utilized lower-resolution tracers or axonal degeneration methods which precluded quantification or detailed anatomical reconstructions. In contrast, the present study enumerates the proportion of CST axons in the corticospinal tracts of the cervical spinal cord, demonstrates the complex topography of individual axons, and indicates the remarkable number of CST axons that cross the midline in the spinal cord itself.

In addition, serial-section reconstructions reveal that individual CST axon branches innervate multiple spinal segments upon emerging from the dorsolateral fasciculus. Individual axon branches can travel in gray matter over rostrocaudal distances of at least 4 mm, elaborating bouton-like swellings at multiple points along their extent. Previous studies using retrograde tracing and electrophysiological methods suggested the presence of such multi-segmental innervation (He et al., 1993; Shinoda et al., 1979); the present study directly demonstrates this phenomenon in individual reconstructed axons.

Based on the present findings, CST axons in the primate spinal cord can be broadly categorized into at least six types (relative to hemisphere of origin): 1) contralateral descent, contralateral termination; 2) contralateral descent, ipsilateral termination; 3) contralateral descent, bilateral termination; 4) ipsilateral descent, ipsilateral termination; 5) ipsilateral descent, contralateral termination; and 6) ipsilateral descent, bilateral termination. This remarkable bilateral arrangement of the primate spinal cord is quantitatively distinct from observations in rats, in which 96–98% of CST projections decussate in the medullary pyramids (Rouiller et al., 1991). Furthermore, rats exhibit only rare CST axonal decussation across the spinal cord midline (Brus-Ramer et al., 2007; Rouiller et al., 1991). In contrast, the anatomy revealed in the present study is likely reflective of human CST organization. Like the non-human primate, humans exhibit substantial recovery of gross and certain fine aspects of movement after spinal cord lateral hemisection (Brown-Sequard syndrome, Little and Halar, 1985; Roth et al., 1991), suggesting the presence of extensive CST decussation at spinal cord levels. On the other hand, complete unilateral lesions to corticospinal projections above the level of the spinal cord (cortex, bulbar projections) are not followed by extensive spontaneous recovery in primates, indicating that, functionally, corticospinal control of movement in primates is primarily driven by activity in the contralateral hemisphere. Therefore, the functional significance of the extensive spinal decussation of CST projections remains to be fully determined.

The bilaterally-projecting and terminating CST architecture may influence spontaneous CNS recovery after CNS injury. For example, direct input from each motor cortex to both sides of the spinal cord may contribute to recovery after unilateral stroke in humans (Kim et al., 2003; Nirkko et al., 1997; Weiller et al., 1993). Reduced inhibition of the intact cortex (across the corpus callosum) could unmask pre-existing connections between the intact

cortex and the affected (ipsilateral) spinal cord (Rocca et al., 2005). Moreover, bilateral terminal arbors arising from single CST axons could provide an anatomical basis for mirror movements of the hands and forelimbs that are sometimes observed after unilateral stroke (Nelles et al., 1998; Nirkko et al., 1997). Nirkko et al. (1997) examined latencies of motor potentials evoked by transcranial magnetic stimulation, and determined that mirror movements were at least partially caused by direct innervation of both sides of the spinal cord by branching CST axons (though the authors could not determine the rostrocaudal level of branching). Thus, the present findings identify candidate anatomical mechanisms that could underlie some forms of plasticity observed after CNS injury.

These studies were performed as part of the development of a primate model of spinal cord injury (SCI). Our description of the bilateral nature of the primate CST and of the presence of spinally-decussating CST pathways highlights a potential target for therapeutic intervention after incomplete SCI. SCI research has generally focused on regeneration of transected axons, but more recently there has been recognition that enhanced sprouting of *spared* axons may be a useful target for promoting functional recovery (e.g., Chen et al., 2006; Glaser et al., 2006; Iarikov et al., 2007; Ji et al., 2006; Massey et al., 2006). The basis for this shift is at least threefold. First, spinal cord injury and other central nervous system or peripheral nerve lesions can induce spontaneous sprouting of nearby systems into territory denervated by the lesion (e.g., Brus-Ramer et al., 2007; Goldberger and Murray, 1974; Kaas et al., 2007; Merzenich and Jenkins, 1993; Murray and Goldberger, 1974; Weidner et al., 2001). Second, it has proven easier to experimentally enhance sprouting of spared axons than to stimulate regeneration of transected axons. Third, most humans who have sustained spinal cord injury suffer complete loss of function below the lesion yet exhibit anatomical sparing of white matter on the spinal cord periphery (Tuszynski et al., 1999); this spared tissue could be targeted for therapeutic enhancement. The latter point is particularly important in light of the present findings because the CST, critical for fine motor control in humans, may retain bilateral projections with only unilateral or asymmetric tissue sparing.

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Figure 1. Biotinylated dextran amine (BDA) tracer injections. 127 injections were performed at 59 sites. The number of injection depths at each site is denoted by asterisk colors: **blue**, one depth; **green**, two depths; **red**, three depths. **A**, **P**, **M**, and **L** indicate anterior, posterior, medial, and lateral, respectively.

Figure 2.

Modified stereological axon quantification method. Black ovals represent axon profiles at the upper face of the tissue section, and blue cylinders represent the remainder of each axon in the tissue section. The counting frame is bounded by green inclusion lines and red exclusion lines. The axon profiles at the upper face must fall within the counting frame or on the inclusion lines, and not touch the exclusion lines. The five axons labeled A–E would not be counted. Axon A is not an axon of passage, because it runs primarily within the plane of section, rather than through it. Axons B and C touch the exclusion lines, while axons D and E are outside of the counting frame and do not touch the inclusion lines.

Figure 3. Coronal brain section from subject B labeled for BDA and Nissl substance. At this anteroposterior location, injections were made into motor cortical areas that innervate the hand and foot (c.f. Fig. 1).

Figure 4.

Corticospinal tracts (CSTs) and arborizations in transverse section. Section from subject A, spinal cord segment C3, labeled for BDA. **A:** Contralateral dorsolateral CST (**Contra. dlCST**). **B:** Ipsilateral dorsolateral CST (**Ipsi. dlCST**). **C:** Ipsilateral ventromedial CST (**Ipsi. vmCST**), plus thinner axons traveling in ipsilateral lamina VIII (**L8**). **D:** Midline-crossing axons near the central canal (**cc**) and in the white matter (**WM**) ventral to lamina X. **E:** Dense arborizations in the intermediate zone of the contralateral gray matter. **F:** Sparse arborizations in the contralateral ventral motor pool. **G:** Sparse arborizations in the intermediate zone of the ipsilateral gray matter. Scale bar: 200 μ m in panels **A–C, E–G**, 133 μ m in panel **D**.

Figure 5.

Corticospinal tracts and arborizations in horizontal section. Section from subject A, spinal cord segments C6–C7, labeled for BDA and lightly Nissl stained. Slightly more ventral portions of the spinal cord are evident in the caudal part of the section. **A**: Contralateral dorsolateral CST. **B**: Ipsilateral dorsolateral CST. **C**: Intermediate zone of contralateral gray matter. **D**: Intermediate zone. **E**: Arborizations in the lateral motor pool. **F**: Ipsilateral intermediate zone. **G, H**: Spinal cord midline (**dashed line**) in the dorsoventral vicinity of the central canal. Note midline-crossing axons. Angular cuts at rostral end of section are fiduciary marks. Scale bars: 125 μ m. Scale bar in **B** applies to panels **A–F**.

Figure 6.

Variability of CST axon diameter. Three CST axons passing through the plane of section. Image was taken from a single, transverse, BDA-labeled section of Subject B, cut on a cryostat set at 40 μm . Image is composite of stack of 10 thinner focal planes, resulting in a dark halo around labeled axons. The thickest axon pictured is 3.6 μm in diameter, the thinnest, 0.9 μm . Axons between 0.5 and 5 μm in diameter were observed. Scale bar: 25 μm .

Figure 7.

Rostrocaudal travel and branching in gray matter. Note the single axon traveling medially through the intermediate zone of the gray matter, bifurcating (**arrow and inset**), and extending branches rostrally and caudally. Image is a composite of a stack of 7 focal planes from a single 30 µm-thick horizontal section. Inset is a single focal plane, 1.5x magnification of main image. **R**, **C**, **M**, and **L** indicate rostral, caudal, medial, and lateral, respectively. Scale bar: 50 µm.

Figure 8.

Serial reconstructions of two axons in segments C8-T1 of subject A (**A**, **B**) and one axon in segments T1-T2 of subject B (**C**, **C'**, **C''**). **C'** and **C''** are higher magnifications of **C**. Axons leave the white matter tracts (**green asterisks**), send one or more branches across the spinal cord midline, and terminate in gray matter. Midline-crossing axons can originate in either the ipsilateral (**A**, **B**) or contralateral tracts (**C**). Branches of a single axon can terminate on different sides of the spinal cord (**green arrowheads** in **B**, **C**, **C'**). The single axon in **C** gives rise to six branches, two of which cross the midline. **C''** shows a more detailed view of the main branch points of this axon; **red** and **blue** tracings represent the two branch systems formed by the initial bifurcation in the white matter. Axons were reconstructed from 96 consecutive transverse sections cut on a cryostat set at 40 μm . One of the axonal branches in **B** is incompletely reconstructed (**red arrow**), as it was still traveling along the spinal cord at the end of the nearly 4 mm series of tissue sections.

Figure 9.

Terminal arbors in the intermediate zone (**A**) and lateral motor pool (**B**) of gray matter. Note the presence of both *en passant* and terminal bouton-like swellings (e.g., **arrows**). Dark halo around the labeled axons caused by compositing of multiple focal planes. **A**: Composite of a stack of 10 different focal planes from a single transverse section from Subject B, cut on a cryostat set at 40 μm and labeled for BDA. **B**: Composite of a stack of 7 different focal planes from a single, 30 μm -thick horizontal section from Subject A, labeled for BDA and Nissl substance. Note the nearby cell body of an α -motor neuron (**MN**). Scale bar: 25 μm .

Raw axon counts and the proportional contributions of each portion of the CST. Counts were obtained in three sections per segment per animal.

Table 1

Animal	Segment	Contralateral dorsolateral tract		Ipsilateral dorsolateral tract		Ipsilateral ventromedial tract		Total
		Axons	% of total	Axons	% of total	Axons	% of total	
A	C3	9079 ± 611	85	1435 ± 105	13	222 ± 43	2	10736 ± 540
	C8	5218 ± 904	83	979 ± 56	16	123 ± 9	2	6320 ± 941
B	C3	7670 ± 1912	90	642 ± 61	8	232 ± 15	3	8544 ± 1971
	C8	6598 ± 1238	87	807 ± 116	11	193 ± 18	2	7599 ± 1364
C	C3	4053 ± 352	89	383 ± 72	8	113 ± 6	2	4549 ± 424
	C8	2207 ± 383	87	261 ± 73	10	75 ± 7	3	2543 ± 399
Mean	C3	6934 ± 2593	88 ± 3	820 ± 548	10 ± 3	189 ± 66	2 ± 0.3	7943 ± 3137
	C8	4674 ± 2246	85 ± 2	682 ± 375	12 ± 3	130 ± 60	2 ± 0.5	5487 ± 2629

Values are expressed as mean ± standard deviation.